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EDITORIAL

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# Gene transfer by viral vect_rs for gen_ therapy

**Key words** Genetherapy · Adenovirus · Herpesvirus · Retrovirus · Thymidine kinase · Cancer · Viral vectors

**Abbreviations** *HSV* Herpes simplex virus

## Gene therapy: from concepts to practice

In a review article Miller [1] reported on the state of the art of human gene therapy as it was summarized in a meeting at the end of December 1991. The human trials in which human genes were successfully transferred in vitro to mark cells of patients and for gene therapy of genetic disorders. The approved gene therapy trials are based on the use of: (a) retroviral vectors and (b) liposomes which contain plasmid DNA that have been found useful in gene transfer into human cells under culture conditions in vitro for administration to the donor of the cell by injection. One of the aims of human gene therapy is to advance anticancer treatment protocols by using human genes whose products have been shown to have suppressive effects on tumor progression. Another aim is to introduce an active human gene which is missing in patients with single gene mutations (e.g., low-density lipoprotein receptor gene transfer to hepatocytes from low-density lipoprotein receptor deficient patients; adenosine desaminase gene transfer to lymphocytes from adenosine desaminase deficient patients [1]).

The crucial elements in gene therapy are the gene delivery vectors. The approved gene transfer trials in human cells utilize retroviral vectors from which all the viral genes are removed or altered and replication of viral genes is supported by cells engineered to provide to the modified retroviral genomes which the viral proteins need for virion budding and for the production of mature virions. This technique has advantages and disadvantages. In the forthcoming volume *Gene Transfer by Viral Vectors for Gene Therapy* [2] we plan to provide a forum for the analysis of developments in the construction and use of viral vectors useful for gene therapy [3–7].

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## Virus vectors carrying human genes into cells from patients under in vitro conditions

### Advantages and disadvantages of viral vectors

The advantage of using retroviral vectors to insert human genes in virus-infected human cells under in vitro conditions is the high efficiency of gene transfer into dividing cells, which depends on the ability of the reverse-transcribed retroviral DNA to integrate into the chromosomal DNA. The high titers of the virions capable of infecting the target cells and the lack of the virus genes for virus replication and egress from the infected cells prevent virus propagation. The inability, however, of retroviral vectors to integrate the viral DNA into the chromosomal DNA of nondividing cells is a difficulty. An additional difficulty in the use of retroviral vectors is the presence of endogenous retroviruses in the human genome at a fairly high copy number (reviewed in [7]), which increases the possibility of the recombination between the retroviral vector and endogenous retrovirus genomes leading to the generation of infectious virus recombinants. It has been shown [1] that the virus progeny of vector-producing cell lines must be carefully tested for replication-competent retroviruses. In addition, cellular RNA can be packaged in the virion, carried in retroviral vectors in virus progeny which is derived from cells that produce the viral proteins (packaging cells), and can be reverse-transcribed in infected target cells. The resulting cellular genes in such cDNA may then integrate into the infected cell chromosomal DNA. The effect of the result-

ing insertional mutagenesis of the cellular genome in the infected cells cannot be easily assessed. Insertion of viral vector DNA near cellular proto-oncogenes may be another possible hazard of the technique. These inherent problems of retrovirus vectors underscore the need for further refinement to improve the vectors for efficient and safe gene therapy procedures. The usefulness of retroviral vectors is addressed in the following four studies [3–6]. Heatlin et al. [3] used ping-pong amplification, a process by which helper-free retrovirions replicated in cocultures of cell lines that package retroviruses into distinct host range envelopes. A most efficient ping-pong vector, pSFF, was developed and used to enhance the expression of the erythropoietin gene in proliferating erythromyoblasts. Richardson et al. [4] summarized recent advances in retroviral gene transfer studies in murine bone marrow cells using vectors containing the human multiple drug resistance (*MDR*) gene. The development and use of safe and efficient retroviral packaging cell lines for transfer of the MDR gene into hematopoietic stem cells and selection of transduced cells in vitro and in vivo is analyzed.

Flamant et al. [5] provided the basic information on vectors derived from avian leukosis and sarcoma viruses and the approach to vector design, including self-inactivating vectors. The concepts for retrovector design may be useful in the development of retrovectors for gene therapy of human cells. Hasegawa and Shimokata [6] used a recombinant retrovirus as a potential vector for human gene therapy to transfer the thymidine kinase gene of herpes simplex virus type 1 (*HSV-1 TK*) to human lung cancer cells. Exposure of cancer cells harboring a functional *HSV-1 TK* gene encoding the viral enzyme thymidine kinase to the antiviral drugs acyclovir and gancyclovir leads to synthesis of the monophosphate form of these drugs by the *HSV-1 TK*, and phosphorylation to the triphosphate acyclovir or gancyclovir is achieved by cellular kinases. These antiviral phosphorylated compounds are inhibitory to the synthesis of cellular DNA in the cancer cells since incorporation of the drugs into the cell DNA will cause inhibition of DNA elongation and therefore prevent cell division and propagation. The study provides a model for gene therapy to control lung cancer cells.

### Synthetic retrotransposon vectors

Chakraborty et al. [9] approached the problem of transcriptional inactivation of the retroviral promoter, due in part to methylation of cpG islands in the retroviral long terminal repeats, by preparing synthetic retrotransposon vectors. These transposon constructs were shown to efficiently transmit and stably insert a neomycin-resistant marker gene into the genome of recipient cells, indicating that safe viral vectors can be developed.

### Viral DNA vectors

Acsadi et al. [7] took advantage of the adenovirus genome which contains large portions of the viral DNA that can be substituted with foreign genes. Adenoviruses have not been reported to be associated with human cancers, and adenovirus recombinants are stable. Adenovirus vectors carrying the 6.3-kb dystrophin minigene under the control of cytomegalovirus or Rous sarcoma virus promoters were constructed and injected into the hindlimb muscles of very young MDX mice. The muscle fibers showed sarcolemmal dystrophin expression.

## Can DNA virus vectors carry genes into the human brain?

The above studies and many more on transfer of genes deal mainly with cell marking and the fate of viral vectors in the rodent host model and in target tissues such as liver and muscle. Recent studies [10,11] on two neurotropic viruses, herpes simplex virus type 1 (HSV-1) and mouse hepatitis virus, revealed that the two viruses spread along different neural pathways from the olfactory bulbs. McLean et al. [12] used HSV-1 as a retrograde transneuronal marker for the central nervous system and found the virus a useful tool in the analysis of neural circuits.

### HSV-1 vector for neuronal gene delivery

Freese et al. [13] developed a defective HSV-1 vector system which provides a method to transfer genes into postmitotic cells such as neurons in adult rat brain. Chiocca et al. [14] used defective HSV-1 mutants 1 ss pathogenic than the parental virus strain to deliver the *LacZ* gene into the rat brain. These studies corroborated by studies from several other laboratories were taken to suggest that "Herpes virus-derived vectors provide a means for the in situ delivery and expression of specific genes into neurons in the central nervous system with little adverse effects on animals" [14].

### HSV-1 vectors may find latent HSV-1 wild-type genomes in brain neurons

Liedtke et al. [15] used a nested PCR assay to detect HSV-1 genomes in trigeminal ganglia and olfactory bulbs which were obtained from 109 human corpses at forensic postmortems. HSV-1 latency was found in 72.5% of trigeminal ganglia and 15.5% of olfactory bulbs. The age-specific prevalence of HSV neuronal latency increases from 18.2% between 0 and 20 years to 100% in individuals older than 60 years. Baringer and Pisani [16] used a similar approach and reported that HSV genomic sequences were PCR-amplified and detected in 35% of the brains, and that the positive brain

areas included medulla, olfactory bulbs, pons, gyrus rectus, amygdala, and hippocampus. The presenc of latent HSV-1 genomes in neurons in various brain tissues in both young and adult human beings may indicate that the use of HSV-1 as a vector for gene therapy in the brain may be needed to prevent the reactivation of latent wild-type viral genomes which can lead to encephalitis and damage to the brain even if acyclovir therapy is administered [17]. Although studies on the presence of latent HSV-1 genomes and traces of other viruses in brain tissue from postmortems of Alzheimer disease patients yielded negative results [18, 19], the use of HSV-1 as a vector to correct the neuronal defect may be considered.

## Conclusions

The search for useful virus vectors and for improvements in currently available retrovectors which may have the capability of transportation by natural transport systems in the human body will open effective ways for targeting human genes to specific cells in tissues in situ. Genetic engineering of virus vectors is a subject of prime importance to the developing gene therapy protocols in humans.

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